DEVELOPMENT OF HIGHLY INTENSIFIED CELL CULTURE PERFUSION MEDIA AND PROCESS WITH TREMENDOUS PRODUCTIVITY POTENTIAL, WHILE HAVING A LOW CELL BLEED REQUIREMENT FOR MAINTAINING AN OVERALL HIGH YIELD

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Process intensification leveraging perfusion offers immense opportunities for yield improvement over fed-batch processes for the production of monoclonal antibodies. In the context of continuous processing, the goal is to achieve highly intensified perfusion processes that allow substantial footprint reduction and enable flexible adaptation in new facilities. Developing a productive and efficient perfusion process requires not only the application of the “push-to-low” concept for reducing the perfusion rate requirement, but also requires in-depth mechanistic development of medium formulations in order to decrease byproduct waste generation, reduce unproductive cell growth and increase productivity. Specifically reducing the usage of cell bleed is particularly desirable for improving the overall yield, since as much as 30% of the generated product may be lost through the use of cell bleed. In this work, we share case studies of perfusion medium development studying classical components such as vitamins and salts that can be manipulated to have profound effect for controlling the cell growth and reducing the use of cell bleed. In one case, the cell bleed rate was reduced down to as low as zero, while still being able to maintain a highly viable culture. Furthermore, in some cases, significant increase in the cell specific productivity (q_p) was achieved when the perfusion culture was switched to a growth suppressed mode. In one example, the q_p increased from 30 pg/cell/day to as high as 115 pg/cell/day when the cell growth was arrested. This led to increased daily volumetric productivities of 3 to 5 g/L/day compared to the control of 1 g/L/day. Cell cycle analysis of the arrested culture by flow cytometry also revealed an induced state of elevated cell population in the G_0/G_1 phase, which is generally considered as the most productive state of the cell cycle. In order to integrate the cell growth control strategy described herein, a two stage perfusion concept is designed where the first stage focuses on rapid accumulation of cells to reach the target cell density, and the second stage switches to a slow growth, yet highly productive and viable perfusion culture.